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Physico-chemical methods of protecting against ionizing radiations

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The primary step when a biological system is exposed to ionizing radiations is the utilization of the absorbed energy in a chemical reaction. Since the energy required to produce a biological lesion is often very small and, moreover since it is initially deposited uniformly throughout the irradiated material, it would seem to be necessary that the biologically important reaction is with a macromolecule. The proportion of biologically active substances of low molecular weight (e.g. A.T.P. or glutathione) which are changed by irradiation with a few hundred roentgens is negligibly small. We assume therefore that the observed lesion is the result of the chemical change of some vital macromolecules present in very limited numbers (e.g. the D.N.A. of the chromosome threads).

In principle there are two general methods of protection:-

(a) A substance can be added which influences the conversion of the energy taken up in such a way that less chemical change occurs in the "vital" macromolecules.

(b) Repair by an added substance of the damage produced in the macromolecule immediately after the primary reaction and before any irreversible change has occurred. Since successive chemical reactions of a molecule activated by absorption of ionizing radiations will occur extremely rapidly, the protective substance bringing about the repair must be present before the irradiation.

Dale was the first to demonstrate protection of the first kind in a system where the action of the ionizing radiations was indirect (i.e. energy absorbed in a solvent produces highly activated molecules which react with the solute; in the case of water it has been clearly established by Dainton, following upon the original suggestion by Risse, that the reactive species are free radicals such as $OH^{\bullet}$, $HO_2^{\bullet}$ and possibly $H^{\bullet}$). Added substances protected enzymes in dilute aqueous solution by competing for the radicals formed in the water which brought about inactivation.

The present paper reports an investigation on the changes produced in a number of synthetic macromolecules under a variety of conditions when protection by different mechanisms was encountered. We hope to be able to establish what type of compounds are most effective in providing protection by the different mechanisms. It may then become possible to deduce by analogy the mechanism of protection in various biological systems by comparing the protective action of a number of substances in vivo with their activity in the synthetic systems.

Competition for free radicals: In Dale's experiments the enzymes were probably inactivated by OH radicals and the activity of the protective agents in these systems is therefore determined by their reaction with OH radicals. We have found that the degradation of polymethacrylic acid in dilute aqueous solution by X-rays is due to HO_2 radicals and have studied the protective action of about 150 compounds in this system. These compounds protect by competing for HO_2 radicals and the protective action is therefore a measure of reactivity with HO_2 radicals. In another system we have studied protection by competition for OH radicals. The order of effectiveness of a series of compounds is not the same in the two systems.

The activity of substances in protecting mice against the lethal effects of X-rays follows closely the HO_2 series and not the OH series. We deduce that competition for OH radicals plays an important part in the protection of mice.

Energy transfer: At first sight it would appear that where the action of the ionizing radiations is direct (i.e. the energy is absorbed by the actual material undergoing change), no protection is possible. According to this view, which is widely held, once sufficient energy has been absorbed by a macromolecule to undergo a chemical change the ensuing reaction cannot be prevented. Nevertheless an experiment carried out by Svedberg and Brohult fifteen years ago pointed to the possibility that direct action was more complex. These workers found that a very specific dissociation of the giant protein molecule haemocyanin into two equal parts could be induced by irradiation with α particles, and that the passage of one α-particle anywhere through the molecule was sufficient to produce this change. This indicated that energy absorbed in one part of the molecule could be transferred to those bonds responsible for holding the two halves together. If energy transfer of this kind can occur, then protection against direct action is theoretically possible.

While studying, in conjunction with A. Charlesby, the degradation of solid polymers (i.e. when the action is "direct") we decided to test this possibility by admixing small quantities of different chemical substances with the polymers. We found that a number of the additives exerted very marked protection (i.e. decreased the amount of degradation produced by a given dose of irradiation). Detailed quantitative studies at present being carried out by D. Toms indicate that the protection occurs by transfer of energy from the polymer to the added substance. Another way of looking at this experiment is to consider that the presence of the polymer potentiates the decomposition of the additive.

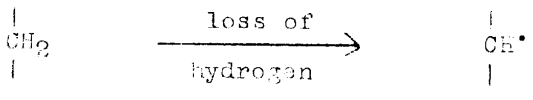
Thus although the energy is originally absorbed uniformly throughout the system, it becomes concentrated in certain points. Energy transfer has also been established in other systems, and of particular interest is the case of co-polymers where energy absorbed by one component is passed along the molecule to the other constituent.

These experiments make it necessary to re-evaluate the data obtained for the sizes of the sensitive volumes of viruses and enzymes from "target area" calculations, since the chemical change may not be confined entirely to the track of the ionizing particle. We have also found that the degradation of both polyisobutylene and polymethyl-methacrylate is greater when the irradiation with γ-rays is carried out at 70°C than at 18°C. A possible interpretation is that energy transfer is promoted by an increase in temperature and that the absorbed energy is therefore utilised more efficiently at the higher temperature. This mechanism may also explain the greater sensitivity to ionizing radiations at higher temperatures which was found by Pollard and his colleagues for dry enzymes.

We believe that the finding that it is possible to protect against "direct action" is of great practical interest since in the cell nucleus vital macromolecules (e.g. nucleo-proteins) are present in high concentrations and direct action must therefore contribute significantly to the total effect. The recent tendency to interpret biological action exclusively in terms of indirect action is, we believe, misguided since the cell does not correspond to the very dilute aqueous solutions studied by radiochemists. We have found with water-soluble polymers that direct action plays a predominant part at concentrations greater than 20%.

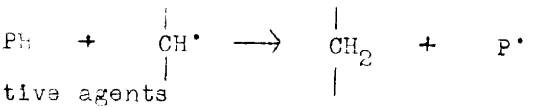
Repair of damaged macromolecule: This type of protection was found to occur when aqueous solutions of polyvinyl alcohol were irradiated. In the absence of oxygen the molecule does not degrade but crosslinks to give a stiff gel. Some added substances, notably -SH compounds, protect by repairing the activated molecule before these can interact to give a polymer network.

The general reaction may be illustrated as follows:



part of polymer molecule — polymer radical

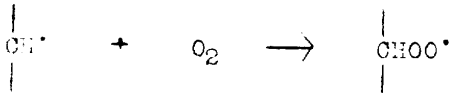
This reaction is common both in "direct" and "indirect" action.



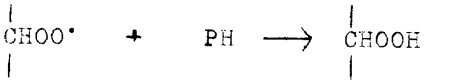
protective agents

The protective agent transfers a hydrogen atom to reconstitute the polymer before the polymer radical has had time to undergo further reactions such as crosslinking.

In the presence of oxygen the polymer radical will be converted to a highly unstable peroxy radical which may decompose leading



to main chain breakdown. This degradation can in principle be prevented by the protector as follows



In this way the polymer is changed but decomposition is prevented by the formation of a stable compound. We have no evidence which indicates that protection of this type plays any part in biological systems.

Inactivation, par les rayons X, d'un agent transformant du pneumocoque

H. Ephrussi-Taylor et R. Latarjet

Le T.P. Sr qui confère au pneumocoque la résistance à une concentration de 2.10^{-3} de streptomycine, a été inactivé en solution aqueuse par des rayons X de 0,7-0,9 A. Les faits suivants ont été mis en évidence :

(a) La courbe de survie est exponentielle, mais présente une cassure pour une survie de quelques pour cent, dénotant une hétérogénéité. Celle-ci ne provient pas de la présence de particules d'ADN héréditairement différentes des autres: les molécules résistant à une très forte dose n'induisent pas chez le pneumocoque la formation de T.P. résistant. Il s'agit d'une hétérogénéité de la solution, actuellement à l'étude.

(b) Le T.P. est extrêmement sensible aux actions indirectes du ravonnement. Tandis que l'extrait de levure à 1 % protège complètement les petits bactériophages contre l'effet indirect, il faut utiliser une concentration d'extrait de 10 % pour atteindre une protection à peu près complète du T.P. contre ces effets. Ce fait est sans doute en relation avec l'absence de membrane protéique

autour de l'ADN, et aussi avec la forme filamenteuse très dissymétrique qui, pour un volume donné d'ADN, offre une très grande surface acceptrice aux radicaux libres.

(c) L'irradiation étant provoquée dans les conditions d'effet direct (notamment sur des solutions congelées), la courbe d'inactivation a fourni pour le poids moléculaire du T.P., une valeur inférieure ou égale à $1{,}12\ 10^6$. Dans ce calcul, les incertitudes liées au groupement des ionisations ont été très diminuées en irradiant conjointement des petits bactériophages de volume connu.

(d) La présence d'oxygène est sans influence notable sur l'inactivation du T.P. Si cette inactivation n'est pas trop éloignée dans sa nature de ce qu'on appelle une radio mutation génique, on peut concevoir que celle-ci serait également indifférente à la présence d'oxygène. Comme les ruptures de chromosomes lui sont très sensibles, on peut penser qu'elles résultent de l'atteinte primaire de substances différentes de l' ADN. L'étude de l'effet oxygène permettrait ainsi de dire si une altération génétique consécutive à l'irradiation, résulte ou non de l'atteinte primaire de l'acide nucléique.

Action préservatrice vis à vis des rayons X de cétones dérivées de polyphénols

A. LACASSAGNE, J.F. DUPLAN, et N.P.BUU-HOÏ

Bien que les polyphénols n'exercent en général aucune action préventive contre le mal des rayons, certains de leurs dérivés tels que les cétones se sont montrés doués d'activité. En continuation de nos recherches antérieures (Lacassagne, Duplan et Buu-Hoï, Comptes rendus 1954/238/1279-81), toute une série de composés nouveaux principalement des cétones dérivées du pyrogallol, des naphtols, de la pyrocatéchine ont été préparés et expérimentés sur la souris dans le but de définir les configurations chimiques qui fournissent des dérivés actifs et qui ralentissent l'absorption de ces corps de telle sorte que leur action préservatrice soit plus étendue dans le temps. Il a été observé que plusieurs de ces cétones sont actives, en particulier celles dérivées du pyrogallol et aussi de la pyrocatéchine. Le 4-béhénoylpyrogallol par exemple, possède un pouvoir protecteur durable.

THE NATURE OF THE PEROXIDE-LIKE SUBSTANCES FORMED IN MICE BY X-RAYS

V.J. Horgan and J.St.L. Philpot

We have estimated oxidizing matter in n-butanol extracts of lethally irradiated mice (1000r of 250 kV X-rays) by an improved anaerobic method using brilliant cresyl blue reduced by a column of cadmium filings. We have confirmed our previous suspicions that part of the oxidised matter is less reactive than tetralin hydroperoxide in that it fails to react appreciably in ten minutes at room temperature but reacts rapidly at 100°C. Similarly slowly reacting matter is present among autoxidation products of isoprene. We think therefore that it may be a cyclic or polymeric peroxide formed by 1, 4 addition to conjugated double bands.

Reactivity studies on autoxidation products of ethyl oleate, linoleic acid, lecithin and mouse extracts made with and without the aid of radiation, have given complicated results which require much further work.

ACTION OF RADIATIONS ON DRY VIRUSES AND ENZYMES

Ernest C. Pollard

The results of the bombardment of a variety of dry enzymes with deuterons of varying energies and fast electrons indicate that the energy which is released by the fast charged particle is confined to the molecule for an appreciable time, and therefore the volume which shows a sensitivity to radiation action is clearly related to the existence of an energy release within the molecular unit.

In view of the fact that some deductions regarding molecular size and shape can be made in this way, the bombardment of viruses by radiations of the same character enables deductions regarding their internal structure to be made. A series of effects have been looked for in bacterial viruses in particular, and from the effects of radiation action deductions regarding the surface structure, the internal structure and the thickness of the membrane surrounding the internal effective part of a virus can be made.

ACTION DES RADIATIONS IONISANTES SUR LES LIPIDES (RÉSUMÉ).

A.CHEVALLIER et C.BURG

L'étude de l'influence des radiations ionisantes sur les lipides est encore à son début.

Les faits se rapportant à l'action "in vitro" se résument dans la formation de radicaux libres dans le milieu contenant les constituants lipidiques, que ceux-ci soient en phase lipidique continue, ou en phase aqueuse sous forme de savons ou d'émulsion. Ces radicaux libres donnent naissance à des réactions en chaîne qui se manifestent finalement par la production de peroxydes, par la conjugaison de doubles liaisons ou par d'autres transformations moléculaires.

Le mécanisme précis des modifications que l'on perçoit n'est pas encore, à l'heure actuelle, pleinement défini. On ne connaît pas, notamment, la nature exacte des radicaux libres qui interviennent, ni les types de réactions en chaîne susceptibles de se produire, en fonction des conditions expérimentales (température, surface, etc). On ne connaît pas les modes de désactivation, et on ignore les réactions que peuvent provoquer les peroxydes eux-mêmes.

Les données sont encore plus incertaines lorsqu'au lieu de s'adresser à des constituants lipidiques dans un milieu pur, on considère des cas complexes où plusieurs constituants différents se trouvent en présence.

Dans ce dernier cas, on peut toujours se demander quelle est la part des interactions entre les différents constituants et les chaînes de réaction déclanchées par les radicaux formés par les radiations ionisantes.

A.- EXPERIMENTATION "IN VITRO".

Les principaux résultats obtenus "in vitro" correspondent aux expériences suivantes:

Depuis longtemps déjà, on sait que l'irradiation des graisses naturelles entraîne l'apparition de phénomène d'oxydation.

Dans plusieurs laboratoires on a obtenu la formation de peroxydes par irradiation d'un grand nombre d'esters d'acides gras en phase lipidique continue: linoléate, oléate, stéarate et tristéarine, et ceci, aussi bien, avec des faisceaux d'électrons qu'avec des rayons X. La présence de tocophérol diminue d'une façon considérable la formation de ces peroxydes.

On a, d'autre part, étudié l'action des rayons X en présence d'eau sur la conjugaison des doubles liaisons de l'acide linoléique en milieu alcalin. Cette conjugaison des doubles liaisons accompagne en général la formation de peroxydes. On obtient un rendement ionique variable suivant les conditions expérimentales, mais toujours très supérieur à l'unité. Il faut remarquer qu'une irradiation, dans des conditions identiques, mais en l'absence d'oxygène, fait tomber le rendement ionique de façon considérable, mettant en évidence le rôle important de l'oxygène dissout.

L'irradiation d'émulsion de linoléate de méthyle dans l'eau a été également étudiée par la même méthode et les résultats obtenus ont été les mêmes. La présence dans l'émulsion de certaines substances liposolubles: tocophérol, vitamine A, vitamine D, inhibe, ou, pour le moins, freine d'une façon importante, la conjugaison des doubles liaisons. On doit en outre remarquer que la présence dans la phase aqueuse de substances

hydrosolubles, telle que la cystéine, l'acide ascorbique ou le glutathion, diminue la conjugaison des doubles liaisons sous l'action du rayonnement X, ce qui semblerait indiquer une interaction possible entre la phase lipidique et les radicaux libres formés dans la phase aqueuse.

L'irradiation "in vitro" des phospholipides n'a pas été très étudiée. On a constaté cependant une décomposition de la lécithine sous l'action des rayons X.

Quant aux différents stérols, ils sont susceptibles d'être modifiés, par les radiations ionisantes. Chacun d'entre eux présente un cas d'espèce.

L'action directe du rayonnement X sur les vitamines liposolubles est très imparfaitement connue. Depuis longtemps déjà on avait constaté que l'irradiation par les électrons et les rayons entraînait un blanchiement du beurre, c'est-à-dire une oxydation du carotène. Par irradiation de la vitamine A et du carotène, dans l'hexane, on a obtenu une destruction de ces deux produits avec un rendement ionique de l'ordre de l'unité. Par contre, l'irradiation in vitro de vitamine dans le sang n'entraîne pas une destruction appréciable de celle-ci. Le rendement ionique, très inférieur à l'unité, implique l'existence dans le sang de substances protectrices. En ce qui concerne le tocophérol et le calciférol en solution dans une émulsion de linoléate de méthyle dans l'eau, on n'a pas relevé de destruction appréciable de ces vitamines pour des doses de rayons X de 1.000 r.

B.- EXPERIMENTATION "IN VIVO".

1) Effets directs.

L'étude des peroxydes formés in vivo dans le tissu adipeux par le rayonnement X a été entreprise dans un certain nombre de laboratoires, à l'aide de réactions colorées très sensibles, telle que la méthode de DUBOULOZ à la thiofluorescéine ou la méthode de GLAVIND et HARTMAN. Les taux de peroxydes obtenus ont toujours été très faibles, très inférieurs aux taux obtenus par irradiation d'esters gras purs. Ce fait s'explique aisément si l'on considère la quantité importante de tocophérol présente dans le tissu graisseux.

Quant au tocophérol lui-même, l'irradiation ne semble pas modifier sa concentration de façon appréciable.

2) Effets indirects.

Lipides totaux.- Ceux-ci correspondent en principe aux modifications constatées au niveau de diverses substances lipidiques appartenant à un organisme vivant soumis à l'action de radiations ionisantes, sans que l'on puisse rapporter les effets constatés à une action directe des radiations.

Le taux global des graisses de l'organisme n'est pas modifié d'une façon importante par les rayons X. Pour tenir compte de l'anorexie consécutive à l'irradiation il est necessaire de comparer les variations du taux global des graisses des animaux irradiés à des témoins soumis au jeûne. Dans ces conditions, chez le rat, le pourcentage total de graisse varie de la même facon chez les témoins et chez les animaux irradiés, au moins pendant les premiers jours consécutifs à l'irradiation.

En ce qui concerne le taux total des acides gras hautement désaturés, à trois ou à quatre doubles liaisons, il reste remarquablement fixe, aussi bien chez les animaux simplement au jeûne, que chez les animaux irradiés.

Résorption intestinale.- L'altération profonde de la tunique intestinale et l'anorexie consécutive ont fait penser pendant longtemps que l'irradiation provoquait une diminution de l'assimilation des graisses. On a pu montrer qu'il n'en était rien, en étudiant, au niveau de l'intestin, la vitesse de résorption d'acides gras à doubles liaisons conjuguées décelables par spectrophotométrie dans l'ultraviolet. On a également déterminé les taux des graisses fécales, chez les animaux irradiés et chez des témoins recevant la même quantité de nourriture que celle absorbée par les animaux irradiés: on n'a pas constaté de différence entre les deux lots d'animaux.

Cependant, la vitesse de résorption intestinale de la vitamine A alcool serait plus rapide chez les animaux irradiés que chez les témoins.

Lipides hépatiques.- L'irradiation entraîne des modifications importantes du métabolisme hépatique, ainsi que le montrent des expériences avec le P^{32} et le C^{14}. Cependant, l'administration d'une dose unique de rayons X n'entraîne pas l'apparition de stéatose caractérisée. L'augmentation du taux de graisses que l'on observe dans certains cas peut sans doute être rapportée à une stéatose du jeûne.

Il en est tout autrement si on administre une dose léthale de rayons X par petites doses quotidiennes. Dans ce cas, on obtient une stéatose caractérisée, aussi bien histologique que biochimique.

Le métabolisme hépatique de la vitamine A semble fortement perturbé par l'irradiation. En effet la vitamine A résorbée par l'intestin, au lieu de s'accumuler dans le foie comme chez les témoins, s'accumule dans la carcasse, sans doute dans le tissu adipeux, et son métabolisme est considérablement accéléré.

L'irradiation entraîne également des modifications du taux de cholestérol contenu dans le foie.

Lipides sanguins.- La constitution lipidique du sang est fortement perturbée par l'action des radiations ionisantes. Chez le lapin, on observe, après l'administration de dose léthale, une opalescence du sérum. On a observé de même des variations du rapport $\frac{\text{albumine}}{\text{globuline}}$, chez le rat et le cobaye. Ces variations disparaissent après extraction du sérum a l'éther et le taux $\frac{\text{albumine}}{\text{globuline}}$ est ramené à la normale. D'après des recherches effectuées par ultracentrifugation, il semblerait que l'irradiation entraîne une accumulation anormale de certaines classes de lipoprotéines dans le sang. L'opalescence constatée chez le lapin après administration de doses léthales serait due à la présence de certaines de ces lipoprotéines

On a relevé, après irradiation, des modifications de la concentration du cholestérol, des phospholipides et de la lipase dans le plasma sanguin.

Enfin, sous l'influence du rayonnement on peut observer des modifications de la constitution lipidique de certains organes ou tissus. C'est ainsi que, notamment, on constate une perturbation dans la nature des graisses au niveau du cerveau et une altération de la synthèse endogène des acides gras au niveau de la moelle osseuse

De tous ces faits, il n'est pas possible de dire quels sont ceux qui sont dûs à une intervention du rayonnement sur les constituants lipidiques eux-mêmes. Il est probable que, parmi eux, certains sont affectés d'une manière directe, d'autres par des voies plus ou moins détournées.

On peut penser que les influences indirectes, que des recherches ultérieures permettront de déterminer, relèvent de l'action des radicaux formés dans la phase aqueuse. Ce problème se complique encore du fait que les constituants lipidiques dans les tissus peuvent, suivant les associations moléculaires qu'ils présentent (lipoprotéines), être classés dans l'une ou l'autre phase.

Cysteamine-cystamine: the possible mechanism of the protective action against ionizing radiation

Eldjarn

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(These investigations were carried out in collaboration with B. Shapiro* and O. Nygaard. The results will be submitted for publication in Cancer Research and Biochemical Journal.)

The possible mechanisms for the protection against ionizing radiations afforded by SH-compounds were recently summarized by Patt (1). He pointed out that the most likely explanations for the protective effect of these compounds are: 1) capture of radicals (or of some oxidants formed during the irradiation), 2) reduction of the amount of oxygen available in the tissue, and hence of the production of certain oxidants (HO_2), and 3) protection or reactivation of sulphhydryl enzymes. However, several workers have claimed that cysteamine does not protect the tissues at the level of the "primary radio-lesions". Thus, cysteamine fails to reduce the x-ray-induced mutations and the male germ cell death in mice (2). The protective effect should rather be due to a stimulation of the regeneration processes (3).

 If the protective effect occurs at the level of the "primary radio-lesions" it must in part be depending on the local concentration of the protective compound during the irradiation. In line with this assumption we have determined the concentration of cysteamine + cystamine (SS+SH) in various tissues 30 minutes after subcutaneous administration of cystamine or cysteamine to rats (8 mg/250 gm body weight). We have used cysteamine and cystamine labelled with S^{35} (4) and isolated the compounds (SS+SH) from the tissue samples by means of carrier-technique.

Thirty minutes after the administration of the compounds the relative concentration of SS+SH in a particular tissue is fairly reproducible and independent of whether cysteamine or cystamine is administered. If the concentration of SS+SH in serum is set at unity, the values found for some other tissues are (averages from 4 experiments): thyroid 4.3, bone marrow 3.2, adrenals 1.9, spleen 1.2, kidneys 1.2, liver 0.3, and testes o.1. These values indicate that the part of the protection which may be ascribed to local capture of radicals or oxidants formed by the irradiation should be greatest in bone marrow, spleen and adrenals, i.e. in organs which are known to essentially determine the radiosensitivity of mammals. The low concentration found in testes may also explain the failure of cysteamine to reduce the x-ray induced mutation and male germ cell death in mice.

If cysteamine-cystamine protect tissue by combining with active radicals or oxidants formed by the ionizing radiation, we would expect certain products of the compounds to be formed. We have investigated the radio-chemical transformations of cysteamine and cystamine in aqueous solutions to check this hypothesis. In this work we have combined the use of heavily sulphur-labelled cysteamine and cystamine (0.2 mc./mg) and analyses by paper chromatography. This technique enabled us to work with cysteamine and cystamine in solutions of the same concentration as that present in tissues after the administration of protective doses of the compounds ($1.4\ 10^{-3}$ M). By measuring the radio-activity on the paper chromatograms we have quantitized the amounts of irradiation products formed (of the order 10^{-5} to 10^{-4} mg). As radiation sources were used a 0.5 curie cobalt source (Co^{60}), an x-ray machine (factors 175 kv., 10 ma. and 0.5 mm copper filter) and also the gamma rays from a 31 mev. pulsed

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* Fellow of the American Cancer Society.

beam betatrone with an on-time of approximately 10 micro-sec. and an off-time of 20 millisec.

Cystamine was formed by the irradiation of cysteamine at pH 3 and 5. The calculated G-values for this transformation were 4-6 and 12-20 respectively. The latter values are in good agreement with those reported for the oxidation of other mercaptans by ionizing radiation (5).

The irradiation of cystamine solutions produced three additional products which were identified as cysteamine, 2-amino-ethane-sulphinic acid and taurine respectively. Our experiments further indicated that the production of the latter two compounds started as soon as cystamine had been formed from cysteamine, i.e. cystamine was able to capture radicals in the presence of cysteamine. However, 2-aminoethane-sulphinic acid and taurine are formed with low ionic yields, the G-values at pH 7.4 being 0.8-1.1 and 0.6-1 respectively.

The formation of 2-aminoethane-sulphinic acid and taurine from cystamine was measured under a number of different conditions. Keeping the total dose constant, the factors varied were dose rate, concentration of cystamine, the acidity and the ionic composition of the solution, temperature, and quality of radiation. Of these factors only the acidity of the solution has a major effect on the yield; the maximum yield was found at pH 7 to 8.

Based on a series of experiments in which the conditions during the irradiation were varied or OH and HO_2 radicals as well as H_2O_2 were produced chemically, we have proposed a mechanism for the oxidation of cystamine by ionizing radiation. The OH radical appears to be mainly responsible for the formation of 2-aminoethane-sulphinic acid, while H_2O_2 seems to produce the greater part of the taurine. The HO_2 radical most probably reduces cystamine to cysteamine with simultaneous production of a cysteamine-S radical and molecular oxygen.

The results so far reported, demonstrate that certain products are formed when cysteamine solutions are irradiated at concentration, pH, and temperature comparable to what are obtained in the animal after the administration of a protective dose. If we could further demonstrate that the same products are actually formed <u>in vivo</u> upon irradiation of a cysteamine-protected animal, this would provide strong evidence that the capture of radicals is in fact an important mechanism by which cysteamine is able to protect the animal organism. Our analytical techniques should permit the study of this very important question.

In preliminary experiments solutions of 1 mg of radioactive cystamine in 1 ml of human serum were irradiated with 800 000 r. We have been able to demonstrate that 2-aminoethane-sulphinic acid and taurine are actually formed in this biological system, although the yield of the product were only about 4 per cent of what obtained in pure water. However, there was a considerable increase in the retention of radioactivity at the starting line of the chromatograms when the irradiated samples were run. The reason for this retention is under investigation. If we assume that this radioactivity represents 2-aminoethane-sulphinic acid adsorbed to proteins, the yield of 2-aminoethane-sulphinic acid + taurine would be 40 to 50 per cent of what obtained in pure water. A yield of this order would mean that 40 to 50 per cent of the active radicals or oxidants formed were captured. This would provide a likely explanation why cysteamine-cystamine under similar conditions <u>in vivo</u> are able to increase the LD_{100} (30 days) dose in mice from 700 r. to 1300 r. total body irradiation.

The results presented above show that the protective effect of cysteamine against irradiation <u>in vivo</u> could well be explained on the basis of their ability to capture the radicals or oxidants formed by

the irradiation. The possibility of an additional effect through the blocking or restoration of SH-groups on vital proteins is not at all unlikely. The relatively large amounts of radioactivity bound by the proteins when cystamine-S^{35} was irradiated in serum may be due to the formation of disulphides between cysteamine and SH-groups of the serum proteins. Less likely is an effect through general lowering of the oxygen tension in the tissues. Some of the contradictory evidences presented in the literature may still be compatible with the radical capture theory if the concentration of the protective substance in a given tissue is taken into consideration. Finally, there are some findings which at present do not fit into the general scheme, notably the effect of shielding the liver during the irradiation period.

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MECHANISM OF MUTATION PRODUCTION IN MICROORGANISMS

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In most organisms the yield of mutations rises linearly with increasing x-ray dose whereas the corresponding response to ultraviolet reaches either a plateau, or a peak followed by a decline with further increases in the dose. Streptomyces is exceptional in that the dose-mutation curves for the two agents are both non-linear, and both show the peaked (or plateau) type of response (depending on the conditions of the experiment) which is normally associated with ultraviolet. This partial loss of the capacity of a cell suspension for induced mutation could be due either to an intercellular (selection) effect, or to the saturation or impairment of some essentially intracellular response. Whatever the factors involved, some at least must be common for both agents, since the effect of combined treatment with ionizing radiation and ultraviolet is less than additive.

The loss is relatively long-lived since, in x-ray fractionation experiments, irradiated spore suspensions showed no measurable return toward normal mutational response to a second exposure, even after an eight hour period of incubation in medium during which a nuclear division had occurred in the (apparent) surviving spores.

 A further similarity between x-ray and ultraviolet mutagenesis is found in the response of Streptomyces spores at different times throughout the first nuclear division and the subsequent development of multinucleate mycelial strands. With both agents the proportion of induced mutants rises by a factor of considerably more than two when the majority of the spores have become binucleate, and declines rapidly as they grow into mycelial strands having four or more nuclei. Under certain conditions the final value is very low. In a typical experiment, with a constant dose of 8000 r of x-rays, the proportion of mutants rises from 20 per cent to a peak at 80 per cent and declines to 4 per cent; and with 200 ergs per sq mm of 2537 Å ultraviolet the values are similar except that the peak is slightly lower.

The association between these changes in mutability and the various nuclear states may perhaps be fortuitious, but so far we have been unable to separate the two. Thus, with incubation in saline few of the spores undergo nuclear division and the characteristic changes in response to x-rays fail to occur. Also, where the saline contains traces of nutrient some of the spores remain uninucleate while others grow into multinucleate strands; under these conditions the characteristic changes in capacity for induced mutation occur but are reduced in magnitude. That the final decline in mutability in such suspensions is a function of the multinucleate strands, and not primarily of the suspension as a whole, is shown by the fact that filtration through very fine filter paper (either before or after x-irradiation), to remove the strands and to leave the ungerminated spores, results in a 3-fold increase in proportion of mutants (from 4.7 up to 15 per cent) thus partially restoring the initial sensitivity.

The resistance of the multinucleate state to induced mutation cannot be due to selective killing of strands containing mutant nuclei, since the survival in some experiments approaches 100 per cent with the standard doses of ultraviolet and x-rays. Also there appears to be a considerable physiological control over the mutability of the strands since, when suspensions are prepared by other than the standard procedure (continuous incubation with frequent agitation), a different response is often observed. Thus with alternate incubation and refrigeration under conditions of

saturation, suspensions were obtained which were consistently sensitive to the mutagenic action of ultraviolet (30 per cent mutation at 100 ergs per sq mm) while remaining insensitive to x-rays (4.5 per cent mutation at 8000 r, mostly of spontaneous origin). And other suspensions, grown under more nearly anaerobic conditions, showed high sensitivity to x-ray induced mutation (34 per cent mutants with 50.1 per cent survival).

Such physiological control could be the result of nuclear selection acting under certain conditions to suppress the induced mutant nuclei. However, unless the mutations induced by the two agents are essentially different, and there is no evidence of this, the direction of selection would have to be determined during a limited period before the cells recover (under the standard conditions of growth prevailing after irradiation) from the transient effects of the particular physiological state and the particular irradiation. Alternatively, the physiological state may well influence the likelihood of induced mutation in individual nuclei.

It should be noted that the alternate incubation and refrigeration also resulted in an accumulation of spontaneous mutants (rising from 0.3 per cent up to 4.1 per cent after 28 hours of incubation) and the factors which prevented the appearance of x-ray induced mutants could not have been operative against these. This does not rule out the possibility of some very complex form of nuclear selection, but it would seem simpler to suppose that there are genuine differences in the capacities of nuclei in different physiological states, to respond to the mutagenic effects of the various agents. If this were true it would be reasonable to suppose that the similarities in the actions of x-rays and ultraviolet involve later steps, and the differences earlier steps, in the chains of events leading to mutation.

The multinucleate strands have also yielded information relevant to the interpretation of the non-linear dose-mutation curves. Samples from the suspension which was sensitive to x-ray induced mutation were exposed to 8000 r and to twice this dose, the per cent mutants with the two doses being 34 and 39 respectively, with 50.1 and 50.5 per cent survival. (The per cent mutants is in each case based on approximately 2700 colonies from irradiated strands; and the strands averaged 8 nuclei each.) The absence of an additive mutagenic effect of the two exposures was similar to that observed in the spores prior to growth, but the high survival precludes the possibility that the non-linear response is due to differential killing of the induced mutant cells.

Originally it was hoped that these experiments would yield information regarding the time of induced mutations. Both x-ray and ultraviolet induced mutations had appeared to be delayed until the time of gene replication in Escherichia coli, since irradiated cells gave rise to colonies sectored for the induced changes even where a "double selection" technique had been used in an attempt to ensure that each colony came from a single irradiated gene complement. Also, the mutagenic effects of ultraviolet in Streptomyces had been found to become stable with respect to light only under conditions favourable to nuclear division (reported elsewhere). However we have not been able to add to these observations any evidence of a critical period in the nuclear division cycle during which mutation might be taking place. Exceptional sensitivity to induced mutation appears to extend over the whole of the binucleate stage, and not just a part of it; and the fractionation experiments show that nuclear division brings no release from an earlier saturation or impairment of the capacity for induced mutation. What appears to have been shown is a striking physiological control over sensitivity to mutation, and we are now in the process of discriminating between the various external factors which may be involved.

Pathology of mice irradiated after injection of β mercaptoethylamine

by M.A. GERSHTZOFF and Z.M. BACQ

There is no doubt that β mercaptoethylamine (cysteamine or Becaptan), injected to mice before a lethal irradiation, confers an effective protection. But the mechanism and even the site of this action is not clear and remains unknown. In what organs does the protection appear? And does it protect the cells themselves or a factor necessary to their regeneration?

In an attempt to obtain an answer to these questions, we have studied the lesions in three radiosensitive organs (spleen, thymus and intestinal epithelium) and in the liver; the importance of this organ in regeneration has been stressed by Maisin and his co-workers.

We have compared C 57 mice subjected to 700 r with or without an injection of 3 mgr of cysteamine just before the irradiation. The detailed results are published elsewhere. Only the main observations will be described here.

For every organ listed above, we have measured the degeneration due to the primary action of X rays, as seen 3 hours after irradiation, and the regeneration observed 3 to 5 days later.

1. Spleen. A. Degeneration. In the lymph nodes of the spleen, the spread of nuclear pycnosis is smaller in mice treated with cysteamine than in control animals. The relation between the intact surface and the total surface of the nodes is, in the mean, 0,151 for untreated mice and 0,371 for treated mice. The difference is quite significative.

B. Regeneration. Four days after irradiation, pycnotic nuclear are very rare in treated animals, but still numerous in some nodes of controls. In these, elimination of degenerated cells and regeneration are slower.

2. Thymus. A. Degeneration. The difference in pycnotic areas is not significative. Pycnosis is massive in both groups of mice.

B. Regeneration. Count of mitosis for 10 microscopic fields gives 48 mitosis for controls and 60 for treated animals. But a statistic study of the results shows that this difference is not significant.

We attribute the uncertain action of Becaptan on thymus to the strong radio-sensitivity of this organ.

3, Intestine. A. Degeneration. There is no difference between treated and untreated mice.

B. Regeneration. The number of mitotic nuclei is 61 for controls, 83 for treated animals. The difference is significant and regeneration is more intense after an injection of cysteamine.

4. Liver. In this organ, the lesions are predominantly cytoplasmic. Their intensity seems to be related to the mode of fixation of the tissue. When the liver is fixed in formalin, there is no difference between treated and untreated mice: 6 hours after irradiation, the cytoplasm of hepatic cells shows a few small vacuoles; 4 days later, the vacuolar state is very pronounced. When it is fixed in formalin and picric acid, there are marked differences between treated animals and controls: in these, the vacuolar state is evident 6 hours after irradiation and very pronounced 4 days later; in cysteamine injected mice, the hepatic cells seem to remain normal.

The vacuolar state of the cytoplasm suggested lipidic degeneration. But an histochemical study showed that, if some lipids were present at the beginning of the degeneration, they disappeared later on. The lack of vacuolar degeneration in Becaptan injected animals when the liver is fixed in a liquid containing picric acid may be related to the fact that this acid is an excellent fixation medium for glycogen. It is possible that the linkage between glycogen (and other polysaccharids) and proteins is fragile in the liver of irradiated animals. But this fragility is greater in controls than in cysteamine treated mice: in these, picric fixation is sufficient to maintain the polysaccharids in the cytoplasm. This observation connects our study with some biochemical researches, particularly those of Fisher.

In conclusion, the injection of βmercaptoethylamine before irradiation effects a direct protection of liver and spleen. It has a strong accelerating action on regeneration in spleen, intestine and probably thymus. Our observations are in favour of the hypothesis that liver is a radiosensitive source of regeneration factors and that protection by cysteamine is effective at the level of some factors concerned with glycogen metabolism.

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THE RELATIVE EFFECTIVENESS OF VARIOUS IONIZING RADIATIONS ON CHROMOSOME BREAKAGE IN TRADESCANTIA

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Previous work[1] on the relative biological effectiveness (RBE) of X rays of several energies, I.2-Mev gamma rays for Cobalt-60 and high-energy beta rays from phosphorous-32, on Tradescantia pollen has been extended to cover the effects of lower energy X rays. Additional studies in which Tradescantia inflorescences are used have confirmed the results of the pollen irradiation, indicating that medium-energy X rays in the 100-kv range are approximately twice as effective as high-energy gamma rays in the induction of chromosomal aberrations. Investigations of the effects of fast neutrons from a cyclotron source on these materials have been carried out and preliminary results show an RBE for one-hit breaks of 8 to 12 relative to medium-energy X rays and 16 to 25 relative to high-energy gamma rays. At the present time only one neutron energy spectrum with a broad maximum in the I-Mev range has been used.

Further work now in progress with a better characterized neutron energy spectrum is expected to lead to a more precise figure for relative biological effectiveness of neutrons.

I. J.S. Kirby-Smith and D.S. Daniels, Genetics 38: 375-385, 1953.

BREAKAGE

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A new interpretation is suggested for "chromatid" ("post-split") changes induced by a diepoxide and by ionising radiations.

It is proposed that several lines of evidence can be most rationally integrated in the hypothesis that this type of change arises only at inter-associations between chromosome parts, and that the aberration itself is always a chromatid exchange. These exchanges can be either between associated parts of different chromosomes, to give the various types of interchromosomal aberration; or between closely neighbouring points on the same chromosome associated by a small loop, to give various types of intrachange, including the two otherwise interpreted as "chromatid" and "isochromatid breaks". It is thus necessarily implied that the single "breaks" seen at metaphase are not the components by reunion of the more complex aberrations, that is, the various interchanges.

It is clear that, if they are interpreted in this way, the observed metaphase changes do not themselves constitute evidence that any breakage process is involved in their formation. On the other hand, the technique employed (the analysis of the consequences of the original change at the later stage of metaphase) cannot provide any information on the actual process of exchange. Therefore the exchange itself is, for the present, regarded quite empirically as the unit of structural change induced. There are, however, other reasons for taking this view. Such an event of exchange occurs as a normal physiologically determined process during meiosis, and appears to be connected in some essential way with the processes of chromosome reproduction. It is therefore proposed secondly that the metaphase changes induced by diepoxide and ionising radiations may most rationally be regarded as heterologous exchanges of the same nature. Since the stages in the process of meiotic chiasma formation which are intermediate between definitive pachytene and diplotene are unknown, it seems justifiable at present to consider the induced exchanges also as empirical entities.

Effect of irradiation on D.N.A. synthesis in regenerating rat liver

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It has been noticed by many workers (notably by V. Euler and Hevesy, Vermund, Barnum et al and Pelc and Howard) and confirmed by ourselves that irradiation of a tissue in VIVO produces a 50% reduction in D.N.A. synthesis which, after large doses (2000 - 9000 r), persists for hours or even days. Far greater doses are needed to reduce the synthesis to less than 50% of the normal.

Various theories have been advanced to explain this phenomenon. It is hoped to produce data obtained from the irradiation of regenerating rat liver at different stages, which may lead to a discussion of these theories. In some of the experiments double labelling of the D.N.A. was used in order to make it possible to consider the synthesis of the whole molecule.

DNA SYNTHESIS IN BONE MARROW STUDIED BY AUTORADIOGRAPHY

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The uptake of ^{32}P orthophosphate, adenine-8-^{14}C and formate ^{14}C into desoxyribose nucleic acid by human bone marrow cells in vitro has been studied using a high resolution autoradiography technique.

Cell suspensions from human bone marrows obtained by sternal puncture were cultured in a liquid medium containing 80% fresh human serum and 20% balanced salt solution. The labelled compounds were added to the culture medium and the cultures were incubated for 2-48 hours at 37°C. At the end of the culture period smears were made, fixed in alcohol, hydrolysed in N HCl 60°C, 6 min, and autoradiographs prepared using the stripping film technique with subsequent staining (after processing the autoradiographs). Differential counts and grain counts were performed on the stained autoradiographs.

The uptake of ^{32}P and adenine ^{14}C indicated that DNA synthesis does not occur throughout the entire intermitotic period of the cells, but only for a limited time (12-15 hours) in the second half of the intermitotic period, finishing about 2-4 hours before metaphase. The total cycle time for the average bone marrow cell was found to be of the order of 40-50 hours.

 Irradiation of the cultures with 5,000r (140kV, 5mA, 1mm Al filter, 15 min) immediately and completely inhibited DNA synthesis of those cells which were in the DNA synthetic period during the time of irradiation. Cells in the first half of the intermitotic cycle developed a latent damage: they could proceed to and enter the DNA synthetic stage, but did not synthesize more than 2-4 hours' worth of DNA, and died off subsequently.

The uptake of formate ^{14}C into DNA has shown the same radiosensitivity as ^{32}P or adenine ^{14}C, but while the two latter compounds have not shown much sensitivity to a folic acid antagonist (aminopterin) the incorporation of formate ^{14}C into DNA was markedly inhibited by 0.5µg/ml aminopterin.

The observations suggest that while the process of assembly of DNA (from base-sugar-PO_4) is sensitive to irradiation it is not sensitive to aminopterin. The incorporation of formate into purines and pyrimidines, however, is inhibited by aminopterin. This also suggests that adenine ^{14}C is mostly incorporated into DNA as adenine, and not after previous decomposition to one carbon compounds.

THE INFLUENCE OF POST-RADIATION FACTORS ON EFFECTS PRODUCED IN BARLEY

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It has earlier been demonstrated that different kinds of damage in barley plants (growth inhibition, chromosome aberrations, sterility, mutation) depend on the water content of the irradiated dormant seeds in the cases of x- and γ-rays. At high water content the radiation sensitivity is smaller, in a manner probably connected with the higher respiration rate. In the case of fast neutron irradiation, no such influence is at hand. A further analysis of the effect has revealed a large plasticity of the moist seeds to post-radiation influences: When the seeds are sown at a low temperature (~ 12°C.) the damage is far more pronounced than at a higher temperature. This difference is not observed when dried seeds are treated similarly.

When the seeds are stored for some days a couple of weeks between irradiation and sowing a reverse influence of the temperature is obtained: the moist seeds develop a higher degree of damage when stored at 25°C. as compared with 12°C. When dried seeds were irradiated, a much smaller effect of storage temperature is found.

When the irradiation is performed with fast neutrons, no effect of germination- and storage temperature on the development of damage is found.

Sur l'apparition, après, action de HN2 - d'éléments polynucléés dans les groupements germinatifs de l'ovaire.

par P. Desaive
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L'ovaire de Lapine soumise à l'introduction intraveineuse d'une dose non mortelle de méthyl-bis (β -chloroéthyl) amine, montre des altérations principalement localisées aux deux extrémités de la chaîne de développement des follicules (stade primordial P et stade préovulatoire G) et est en outre le siège de la formation de nombreux groupes de type plasmodial, constitués par des ovocytes réunis à l'intérieur d'une enveloppe folliculaire commune

La présente étude est destinée à préciser l'origine de ces formation et à déterminer le rôle que peuvent jouer, à leur égard, les radioprotecteurs chimiques.

THE RELATION OF DOSE AND MITOTIC STAGE AT TREATMENT TO X-RAY INDUCED STICKINESS OF CHROMOSOMES

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Grasshopper neuroblasts in hanging-drop preparations were X-rayed at known mitotic stages and examined for evidence of stickiness at short intervals of time following treatment. Stickiness is manifest in the adherence of different chromosomes (clumping), as detected at prometaphase, metaphase, and anaphase, or in the failure of sister chromatids to separate normally at anaphase. Of the mitotic stages studied, namely, very late prophase, prometaphase, metaphase, and anaphase, the earlier the stage of the cell at treatment the smaller the dose needed to produce a given effect. This different susceptibility may be interpreted either as resulting from the length of time available for stickiness to develop between treatment and observation or as a different degree of sensitivity of the chromosomes at different stages of mitosis.

COMPARISON OF THE PHYSIOLOGICAL RESPONSE TO RADIATION AND TO RADIOMIMETIC CHEMICALS

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 The effects of whole body X-irradiation on the body weight and on the blood of rats have been compared with the effects produced by two types of radiomimetic chemicals. One of these "Myleran", 1:4-dimethanesulphonyloxybutane $CH_3.SO_2O-(CH_2)_4-O.SO_2CH_3$ produces little immediate weight loss but can cause a delayed weight drop at about 12 to 14 days after a single dose. It has little effect on the circulating lymphocytes but produces a steady fall in the neutrophils (polymorphs), which reach a minimum at about 14 days. The larger doses produce a rapid fall in erythrocytes and platelets after about 8 to 10 days corresponding with a general haemorrhagic state, and the delayed weight drop is almost certainly the result of the anaemia thus produced. With toxic doses death occurs at about 10 to 12 days as a result of massive haemorrhage. On the other hand the "nitrogen mustard" derivative N-N-di(2 chloroethyl)p-amino-phenyl γ butyric acid. $p.HOOC-(CH_2)_3-C_6H_4-N(CH_2CH_2Cl)_2$ produces an immediate weight loss lasting 2 to 3 days followed by recovery of normal growth rate. Its effect on the blood is to cause a rapid fall in lymphocytes to a minimum at about 2 to 3 days followed by a gradual recovery. There is also a rapid fall in neutrophils with a very rapid recovery with considerable neutrophilia at about 8 to 10 days quickly returning to normal. With toxic doses death nearly always occurs during the period of initial weight loss.

Thus Myleran shows mainly the myeloid effects of X radiation whilst the nitrogen mustard shows mainly the lymphoid effects. A combination of equal amounts of the two chemicals (12.5 mg/kg. of each) gave weight and blood effects extremely similar to those produced by a single dose of 200 r whole body X-irradiation.

STUDIES ON THE MECHANISM OF RADIATION PROTECTION AND RECOVERY WITH CYSTEAMINE AND MERCAPTOETHANOL

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In comparing the effectiveness of β-mercaptoethylamine (cysteamine), and β-mercaptoethanol (MCE) in protecting E. coli, B/r, against X radiation, it was found that at 60,000 r cysteamine reaches a high plateau level of protection at about 0.006 M whereas the MCE protection curve flattens out at a lower level when a concentration of 0.1 M is reached. Cysteamine gives remarkable protection against radiation up to 60,000 r (dose reduction factor - DRF = 12). At increasing energy >60 x 10^3 r, the protection falls to a considerably lower level (DRF = 6). In contrast to this, MCE protects uniformly with increased energy at a somewhat lower level than the first compound (DRF = 8). The efficacy of cysteamine in protecting against X-ray damage is reduced significantly by the presence of phosphate salts, a phenomenon which does not occur with MCE; furthermore a significantly lower concentration of phosphate as compared to cysteamine is required for this effect.

A solid (agar) growth medium which contains yeast extract, beef broth, or some other natural extract used after irradiation is necessary to bring out the protection of cysteamine. However, a synthetic medium (inorganic salts, glucose, glutamate, uracil, and guanine) can also serve as an effective growth medium for cysteamine protected bacteria. This is the same medium which promotes partial recovery of unprotected, irradiated E. coli (Stapleton et al.). A growth medium consisting of inorganic salts, glucose, and agar which will support growth of nonirradiated E. coli will support, sparingly, growth of E. coli irradiated in the presence of cysteamine reducing the DRF from 12 to 2; whereas E. coli irradiated in the presence of MCE will grow on this simple medium rather well, changing the DRF only from 8 to about 6. When E. coli is irradiated in a phosphate buffer solution, where reduced protection is obtained with cysteamine, the growth requirements are somewhat less fastidious.

Adding MCE or other protective agents to cysteamine during irradiation at levels below 60,000 r will not produce additional protection. However, at levels in excess of 60,000 r where, as mentioned above, the effectiveness of cysteamine is reduced, MCE as well as other protective agents, will add readily to the protective ability of cysteamine. The direct effect of irradiation is not on the cysteamine since cysteamine irradiated with energy levels of X ray even up to 120 x 103, behaves for all practical purposes like non-irradiated cysteamine.

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THE EFFECT OF RADIATION ON FROZEN TUMOUR CELLS

by

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It has been shown that the radiation effect can be influenced by the metabolic activity of irradiated biological objects. In tadpoles the radiation effect was delayed if the animals were chilled during and after exposure. (Spear and Glucksmann, 1939.) Goldfeder (1951) comparing the radiosensitivity of histologically similar mouse tumours found the radiation effect increased in those showing a lower metabolic rate. As similar result was obtained with the S37 tumour exposed as ascites and subcutaneous form (Lasnitzki, 1953). For the ascites form which according to Warburg and Hiepler (1952) functions at very low energy levels the MLD was found to be 1/5 of that for the solid sarcoma.

In the present work the effect of radiation was compared on tumours in the fresh and frozen state at which metabolic activity should be at a standstill. The tumour used was the Ehrlich ascites tumour which, as a rule, preserves its viability if frozen and stored at the temperature of dry ice, i.e. - 79°.

Ascites fluid containing the tumour cells was divided into four equal batches two of which were frozen at - 79°. Of the remaining two, one was irradiated in vitro at room temperature and injected subcutaneously, the other was inoculated without exposure to serve as control. The frozen cells were kept at - 79° for 7 days one batch was then exposed, thawed and inoculated immediately after exposure, the other thawed and injected as control. In one set of experiments gamma rays were used and exposure took place at room temperature, in another, x-rays were used and the frozen cells kept on dry ice during exposure. In both experiments the dose was 1500 r at 75 r/min. The radiation effect was measured by the number of subcutaneous tumours obtained from control and irradiated grafts.

The number of tumours obtained from fresh and frozen control grafts is of the same order in both experiments, amounting to a percentage take of 80-90%. This indicates that the viability of the cells remained unimpaired by freezing alone. A comparison of the percentage of irradiated fresh and frozen implants shows that the appearance of tumours is delayed and their number significantly reduced in cells derived from cells exposed in the frozen state. In the gamma ray experiment irradiated fresh cells produced tumours in 34% of the mice two weeks following inoculation but none appeared in mice injected with frozen irradiated cells. At four weeks the percentage take was 56% for irradiated fresh cells and 22% for irradiated frozen cells. At this time the tumour volume derived from irradiated frozen cells was one fifth that from irradiated fresh cells. Cell counts made in the growing edge of tumours showed that the mitotic rate was similar in both types of tumours but that the proportion of abnormal meta and anaphases was increased threefold in tumours derived from irradiated frozen cells. In the x-ray experiment in which the frozen cells were kept on dry ice during exposure a similar greater effectiveness of radiation on frozen cells was obtained. At two weeks following inoculation the take was 42% for irradiated fresh cells and 10% for irradiated frozen cells, at four weeks it was 48% for irradiated fresh cells and 18% for irradiated frozen cells.

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Chromosome breakage by di(2:3-epoxypropyl)ether and by X-rays in Tradescantia roots.

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Chromosome aberration induced by di(2:3-epoxypropyl) ether in the root cells of *Vicia faba* occur largely in the demonstrable heterochromatin and the reunion involved is between chromatids, i.e. chromatid reunion (R') and sister reunion (SR). The effect of this substance and of X-rays on the meristematic cells of *Tradescantia* roots is being tested. This material is sensitive to di(2:3-epoxypropyl)ether and after a period of mitotic suppression considerable numbers of chromosome breaks appear in spite of the lack of demonstrable heterochromatin. Reunion appears to be entirely of the R' and SR types in contrast to that following treatment with X-rays when chromosome reunion (R") is frequent in the root cells at the same time interval after treatment. Study of the types of aberration, their frequency and location in this material has an important bearing on their mode of induction.

INFLUENCE OF OXYGEN ON RADIATION DAMAGE TO MICROORGANISMS

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A number of bacteria representing strict and facultative aerobes and anaerobes, as well as some yeasts, were exposed to X-rays (190 Kvp, no added filtration, dose rate 6500 r/min.) at doses from 6.5 to 65 x 10^3 r. Known cell concentrations of washed cells were irradiated in phosphate buffer + glucose in absence of any added growth-promoting substance. During irradiation the liquid phase was either free from dissolved O_2 (the gas space containing N_2 or H_2) or it was in equilibrium with O_2 of decreasing percentage (100% O_2; 20% or 5% O_2/N_2). In some experiments with 20% and 5% O_2 the nitrogen was replaced by carbon monoxide.

Immediately after irradiation a known amount of cells was transferred either into fresh buffer + glucose or into a nutrient medium in manometer flasks and the rate of some metabolic processes measured for up to 10 hours. These included: O_2 uptake, CO_2 production, aerobic and anaerobic fermentation or acid production and utilisation of H_2 (vibrio desulphuricans). Under these conditions the increase, for example, in O_2 uptake or anaerobic fermentation with time is proportional to the increase of dry weight of bacteria, i.e. it is a true reflection of growth.

Most irradiations were done with 6.5, 13.0 and 26.0 x 10^3 r (1, 2 and 4 min. irradiation). The main results are as follows:

1. O_2 uptake, CO_2 production and anaerobic fermentation or acid production were not significantly affected, when measured in washed non growing cells. Aerobic fermentation of baker's yeast was slightly inhibited.

2. Growth inhibition became apparent only after a lag period which is the shorter, the higher the X-ray dose.

3. All growing cells showed an "O_2-effect" on irradiation. The relative sensitivity for O_2-treated as compared to N_2-treated cells was approximately three-fold for some cells (Staphylococcus albus, Lactobacillus Delbrückii). On the other hand a strictly anaerobic organism (vibrio desulphuricans) was unaffected by 6.5 x 10^3 r in N_2 but almost completely inhibited by the same dose in air; and the growth of vegetative forms from spores of B. subtilis was only inhibited after irradiation (26 x 10^3 r) in presence of O_2 but not in N_2.

4. In order to study whether the "O_2-effect" is dependent on a particular metabolic state of the cell or a particular equilibrium of an enzymic system involved in respiration, cells were irradiated in presence of O_2 while their respiration was inhibited by respiratory poisons. Thus, in the case of one bacterium, namely Sarcina lutea, it has been possible at an X-ray dose of 26,000 r to almost completely suppress the O_2-effect, i.e. the cells, after removal of the poison, behaved as if they had been irradiated in N_2. The effective inhibitors were CO, KCN, hydroxylamine and azide. Urethane did not abolish or diminish the "O_2-effect".

The implication of this result will be discussed. It is suggested that it favours the conclusion that the "O_2-effect" is due to a reducing and not to an oxidizing agent.

A few experiments were also done with irradiation of some enzymes or enzymic systems, namely

a) glucose oxidase (notatin), irradiated + glucose;
b) Heart-muscle preparation (Keilin & Hartree, (1),) irradiated ± succinate;
c) Muscle mitochondria.

In neither case had irradiation up to 32,500 r (no higher dose was tried) any effect on the rate of subsequent oxidation of glucose,

succinate or α-ketoglutarate respectively; nor was there any effect on oxidative phosphorylation by irradiated mitochondria. The significance of these findings will also be briefly discussed.

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PHOSPHORYLATING ACTIVITY OF MITOCHONDRIA AFTER TOTAL BODY IRRADIATION

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Our studies were initiated with the object of collecting information on the effect of ionizing radiation on metabolic processes in the cytoplasma. It was postulated that the well-known interference with cell division and D.N.A. synthesis which follows exposure to ionizing radiation might well be the result of damage to biochemical reaction-syatems outside the nucleus. Since D.N.A. synthesis is generally supposed to be dependent on energy generating processes which occur in the cytoplasma, the oxidative phosphorylation of mitochondria seemed to present the most obvious subject for investigation.

In preliminary experiments with mitochondrial preparations from various tissues, it was found that spleen mitochondria showed a decreased phosphate uptake after total body irradiation. Most of our subsequent work has been carried out with rat spleen tissue, because of its radiosensitivity and its relative abundance per animal. Our experiments are usually performed at 4 hours or less after total body irradiation, because after a larger interval the structural changes in the spleen are so radical, that the study of biochemical reactions in these altered tissues cannot in our opinion be expected to throw much light on the mechanism of initial radiation injury.

A decrease of oxidative phosphorylation of isolated spleen mitochondria following total body exposure to X-rays has now been reported by several investigators. In 1952 Potter and Bethel (Federation Proc.,11,270.) described a decrease of phosphate uptake by mitochondria isolated from rat spleen at 1 to 24 hours after total body irradiation with 800 r. Similar observations were reported shortly afterwards by ourselves (Trans. Faraday Soc.49, No.363,1953). In these experiments rather large doses of X-radiation, namely 800 r and 1100 r, were administered to rats and spleen mitochondria were isolated 2,4 and 24 hours afterwards. In addition to a diminished phosphate uptake, some decrease of oxygen consumption was observed in most experiments, although the latter effect has been generally of lower magnitude. Thus a decrease of P/O ratio's has been consistently found with several substrates. Maxwell and Ashwell (Arch.Biochem & Biophys.,42,389,1953) have published comparable results obtained with mitochondrial preparations from mouse spleen at 1 to 7 days after a lethal dose of total body X-irradiation.

After the effect of large doses of total body irradiation had been established, we have attempted to assess the sensitivity of oxidative phosphorylation to this type of injury. Furthermore our studies have been extended to mitochondria isolated from rat thymus. The methods employed with spleen mitochondria have been described previously (Brit.J.Radiol. 27,127,1954) and were applied with minor modifications to the study of mitochondria from thymus. The data obtained show that in the case of spleen mitochondria, oxygen uptake is usually far less affected than phosphorylation, while in the case of thymus mitochondria this difference is not so outspoken, although P/O ratio's are depressed after irradiation in most experiments.

The minimal doses of radiation which are capable of inducing a decreased mitochondrial phosphorylation were found to be between 50 r and 100 r for both tissues. Microscopical examination of the tissues used in these experiments revealed extensive cellular destruction at 4 hours after the larger doses. In the spleen after a dose of 50 r, some cell débris is present in most of the lymph follicles, while in an occasional one very little if any sign of damage is to be found. After the 100 r dose a greater number of nuclear fragments and pycnotic nuclei have been observed, but the destruction is markedly less than after the larger doses of radiation.

In thymus slices only a small amount of nuclear fragmentation could be observed at 4 hours after 50 r and many mitotic figures were present. After 100 r the signs of destruction were more extensive, although many apparently normal nuclei and a few mitotic figures remained. From this it appears that the minimal doses of total body irradiation required to produce morphological evidence of nuclear damage and those necessary to elicit the biochemical change under discussion do not differ widely if at all.

In this connection it is perhaps of interest to mention that we have not been able to observe any interference with the oxidative phosphorylation of mitochondria isolated under similar conditions from the livers of irradiated rats. Even doses of 5000 r administered to the liver region fail to produce an effect as observed in the case of spleen and thymus mitochondria. These observations suggest a relation between the latter effect and the radiosensitivity of the cells from which the mitochondria are derived.

The phenomenon has also been observed with mitochondrial preparations from spleens which were exteriorized during irradiation while the rest of the animal was being shielded. The depression of phosphorylating activity after total body irradiation is therefore at least for the greater part the result of the action of the X-rays on the spleen tissue itself.

In vitro irradiation of isolated spleen mitochondria, both in the inactive state at 0°C. and during incubation in the presence of substrate at 38°C., has uniformly yielded negative results, which is in accordance with observations of Potter et al.

The results obtained with rat spleen do not allow an evaluation of the radiosensitivity of the mitochondria in the various haematopoetic cells, since this organ contains beside lymphoid elements, also variable quantities of erythropoietic and myelopoietic cells. Our observations on the thymus infer that the mitochondria of lymphoid cells are affected by total body irradiation. In order to assess the sensitivity of mitochondria from erythropoietic cells in this respect, an increase of the red cell forming elements in the spleen has been induced by exposure of the rats to intermittant hypoxia for a 3 to 4 day period. Mitochondrial suspensions prepared from the spleens of these rats were found to exhibit much higher rates of oxidative phosphorylation, with a concomitant increase of P/O ratio's to nearly double the control values. This activity is also severely depressed after total body irradiation, which seems to justify the conclusion that the phosphorylating activity of mitochondria from erythroblastic cells is also sensitive to irradiation.

The work so far summarized has been of a descriptive kind. The significance of the disturbance of oxidative phosphorylation with regard to the mechanism of radiation injury to the cell is not known. Our results indicate, however, that the sensitivity of this process to radiation is comparable to that of the nuclei in the same tissues.

The nature of the derangement of oxidative phosphorylation is still obscure. There have been suggestions that it might be secondary to the increased ATP-break-down which occurs in spleen homogenates following total body irradiation. In 1952 Ashwell and Hickman (Proc. Soc. exp. Biol. & Med., 80, 407.) reported a threefold increase of ATP dephosphorylating activity (to be denominated ATP-ase activity hereafter) of mouse spleen homogenates at 1 to 11 days after total body irradiation with a lethal dose of X-rays and in 1953 more details on this subject were published (J. Biol. Chem., 201, 651). The authors conclude

that this rise could be best explained by the premise that a large amount of inert cellular material had been destroyed after irradiation, while the particular enzyme systems were unaffected. More recently Dubois and Petersen (Amer. J.Physiol., 176, 282,1954.) showed that the increase of ATP-ase activity could be observed in rat spleen and thymus homogenates even after total body irradiation with doses as small as 25 - 100 r and they suggest the possible significance of this effect with regard to the maintenance of energy requiring reactions after irradiation. Previously it had been pointed out by Maxwell et al. that the increase of ATP-ase activity cannot be the cause of the depressed phosphorylation of spleen mitochondria, because ATP-ase activity had been blocked by NaF in the system used for the measurement of oxidative phosphorylation. In our opinion this argument could not be considered satisfactorily because the amount of NaF employed by Maxwell et al. leaves a small part of the ATP-ase activity uninhibited and we found this remaining activity in homogenates to be proportional to the values obtained in the absence of NaF. A more extensive investigation of the possible role of ATP-ase in the disturbance of oxidative phosphorylation has therefore been carried out. The results which confirm the conclusion of Maxwell et al. may be summarized as follows:

(1) The increase of ATP-ase activity of spleen homogenates is not apparent within a few hours after irradiation, when the disturbance of oxidative phosphorylation of the mitochondria is already well developed.

(2) The ATP-ase activity of isolated spleen mitochondria after total body irradiation has been found to be normal, in the presence of a severely impaired phosphorylating capacity of the same preparations.

The rapidity with which the depression of oxidative phosphorylation appears after irradiation relative to other biochemical changes is noteworthy. The decrease of anaerobic glycolysis which has been described in mouse spleen homogenates after total body irradiation by Hickman and Ashwell (J.Biol. Chem., 205,651, 1953.) was found by us to appear several hours after the disturbance of oxidative phosphorylation had been well established, in the case of rat spleen.

In conclusion, the evidence which has been collected indicates that the decreased phosphorylation of spleen mitochondria after total body irradiation is probably caused by some block in the oxidation coupled phosphorylating reactions, the exact nature of which is as yet little understood.

SOME FACTORS CONTROLLING THE HAEMATOPOIETIC REGENERATION IN WHOLE-BODY IRRADIATED RATS

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Many works in the field of Radiobiology have shown that there is a close relationship between the survival rate of whole-body irradiated animals and their ability to regenerate the destroyed haematopoietic tissues.

During recent years it has been shown that different chemicals are able to protect animals against a lethal dose of X-rays. Whereas animals submitted to a lethal whole-body irradiation do not show any regeneration of their haematopoietic tissues, there is an extensive regeneration of bone marrow, spleen, lymph nodes and thymus in the animals receiving a protective agent before the lethal irradiation. Quite similar results have been described in animals protected with potassium cyanide (Betz), glutothione (Cronkete et al.), thiourea (Mole), cysteine (Lorenz). An identical stimulation of haematopoiesis is observed in animals protected by spleen- or bone-marrow homogenates injected after X irradiation (Jacobson et al.; Lorenz et al.).

In former experiments, we have shown that, in animals submitted to a lethal dose of X-rays, there is an inhibition of haematopoiesis which is independent of the tissular lesions themselves. It is possible, indeed, by grafting the spleen of an irradiated mouse to a normal one to induce an extensive regeneration of myeloid and lymphoid tissues within the graft. Such a regeneration would never have taken place if the splenic tissue had remained within the irradiated body till death. From these observations, we may conclude that the regeneration of the haematopoietic tissues depends not only on tissular lesions but also on the humoral conditions within the body where such tissues are living. These observations led us to investigate different factors which could possibly control the haematopoietic regeneration of irradiated rats.

The first factor we started to investigate was the influence of the adrenal cortex. It is well known that whole-body irradiation stimulates the activity of the adrenal cortex (Patt et al.). The increased protection of adreno-cortical hormones could influence the haematopoietic regeneration; Barker and Ingle have observed indeed an atrophy of bone marrow in rats treated with large doses of cortisone and A.C.T.H.

Influence of cortisone and desoxycorticosterone acetate (D.C.A.) on haematopoietic regeneration after a sublethal dose of X-rays.

These experiments are made in order to see whether adrenocortical hormones are able to inhibit the haematopoietic regeneration, after a sublethal irradiation (500r). The control rats irradiated only have, indeed, an extensive and early regeneration of thymus, spleen, lymph nodes and bone marrow. The repair of the destroyed tissues starts on the fourth day after irradiation.
Daily injection of 5 mg. of cortisone acetate to rats irradiated with 500r. does not influence the regeneration of spleen, lymph nodes or bone marrow; the regeneration of the thymus only is inhibited by such a dose of cortisone. On the contrary, the injection of 10 mg. of cortisone daily does block the regeneration of spleen, lymph nodes and bone marrow as well as the repair of thymus. The histological picture of the haematopoietic tissues of these rats is quite similar to that observed in rats receiving a lethal irradiation of 800r.

The daily injection of 3 or 5 mg. of D.C.A. does not modify the regeneration of haematopoietic tissues.

Influence of adrenalectomy on the haematopoietic regeneration.

Adrenalectomized rats irradiated with a sublethal dose of X-rays (500r.) show the same haematopoietic regeneration as normal rats irradiated in the same conditions.

Adrenalectomized rats are very sensitive to a lethal dose of X-rays (800r.) and die before any regeneration could possibly take place. Therefore, we used adrenalectomized rats supplemented with a dose of D.C.A. (3 mg. daily) or cortisone (2.5 mg. daily) too small to influence any haematopoietic activity. The resistance of these rats appeared to be normal. Although any possibility of hypercorticism was excluded, no haematopoietic regeneration has been observed in these rats.

The results of these experiments are not conclusive. Should an increased adrenocortical secretion be able to inhibit the haematopoietic regeneration, there is no doubt that the hypercorticism following a lethal whole-body radiation is not the only factor involved in the inhibition of haematopoiesis. The adrenalectomy does not succeed, indeed, in stimulating the haematopoietic regeneration of irradiated rats. Some other factors must be involved in the regulation of this phenomenon.

Recently Selye has shown that somatotrophic hormone is able to counteract the catabolic effect of protein which normally occurs after a stress or an injection of cortisone. On another hand, it is known that the production and differentiation of blood cells is closely related with the protein metabolism. Therefore, we studied the effect of somatotrophic hormone on the haematopoietic regeneration of irradiated rats: We compared the results with those obtained by using other substances such as testostérone propionate and Vitamin B12 whose effect on protein anabolism is also well known.

Influence of somatotrophic hormone on the haematopoietic regeneration

Three groups of rats are studied, all irradiated with a lethal dose of X-rays (800r.). The first group is used as control. The animals of the second group are irradiated and injected daily with 5 I.U. of growth hormone. The rats of the third group are adrenalectomized before the irradiation; afterwards they are injected daily with 2.5 mg. of cortisone and 5 I.U of growth hormone.

In the control group, all the animals died within the eleven days following the irradiation, without showing any sign of haematopoietic regeneration. Those rats irradiated and injected with growth hormone behaved like the controls: they died between the fourth and the tenth days without regeneration of their haematopoietic tissues. On the contrary the adrenalectomized rats injected with somatotrophic hormone proved more resistant to a lethal dose of X-rays: about 25% of the individuals of this group survived, showing an extensive regeneration of lymph nodes, spleen and bone marrow.

Influence of testostérone propionate and Vit.B12 on the haematopoietic regeneration.

The two substances used in this experiment have the same action as growth hormone: they are quite inactive when injected to normal irradiated rats, while they do, on the contrary, stimulate the haematopoietic regeneration of adrenalectomized animals; here again, about 20% of the individuals survived to a lethal whole-body irradiation.

From these experiments, we may conclude that substances stimulating the anabolism of proteins are active in stimulating the haematopoietic regeneration of irradiated rats provided they are given to adrenalectomized animals. They are quite inactive in normal rats. These results indicate that, as far as haematopoiesis is concerned, there is an antagonistic effect of adrenal hormones and substances like growth hormone, testosterone propionate and vitamin B12. It is likely that such an antagonism is related to the action of the substances on the metabolism of proteins.

In whole-body irradiated rats, hypercorticism plays a role in the increased catabolism of proteins which is observed in the animals. Betz and Jehotts have shown that a lethal dose of X-rays produces in the rat a quick reduction of food intake, a reduction of nitrogen excretion, together with a negativation of the nitrogen balance. After five days, a secondary increase of the nitrogen excretion takes place with a still more marked negativation of the nitrogen balance. Adrenalectomy prevents the secondary increase of nitrogen excretion although the nitrogen balance remains negative.

Our results confirm the relationship between the regeneration of blood cells and the metabolism of proteins. The disturbances of this metabolism which follow a whole-body irradiation are poorly understood. Besides the hyperactivity of the adrenal cortex, there are other unknown factors interfering with the nitrogen metabolism. A better knowledge of these factors would probably be a great help in the discovery of the factors which control the haematopoiesis of the irradiated body.

The "New Moon and Sixpence" Skin Test in Radiobiological Studies

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A variation in technique in the course of investigations on diffusible substances, the reciprocal vicinity effect of irradiated tissues and the unirradiated neighbourhood on the degree of radiation reaction in which adjacent square areas separated by varying widths of normal skin (Jolles 1, 2, 3) were used, led to the development of a skin radiation test for screening of protector substances, tracing the whereabouts of radioactive phosphorus injected intradermally, the study of spreading factors and other radiobiological investigations.

When a dose of X-rays is given to circular areas of varying diameters surrounded along half of their circumference at a distance of 0·5-1·0 cm. by an 0·5-1·0 cm. wide "new moon" shaped strip the skin reaction of the lower half of the circle, i.e. not having in its vicinity an irradiated strip of skin, shows a degree of reaction which is less than that on the upper half of the circle and on its neighbouring strip. The potentiation effect due to the reciprocal vicinity of irradiated areas, and the decrease of reaction on the sectors surrounded to a greater extent by normal tissues, are not accounted by the minimal variations in dose actually delivered to different parts of the treated areas as measured by Sievert chambers.

Quantitative data can be obtained from readings of the intensity of reaction at different sectors of the irradiated areas of skin by means of an erythemameter specially constructed to the author's specification by Lovibond Tintometer Ltd., which enables one to assess accurately the hue, brightness and saturation of the erythema and plot colour changes in a diagrammatic form without the need for visual memory and vague descriptions of readings on successive days, or after a period of time.

Screening of "Protector" substances

A series of specimens of substances not identified except for their molar weight, and labelled A to G, were obtained through the kindness of Professor A. Haddow and Dr. P. Alexander of the Chester Beatty Research Institute. This series included substances which have no protective action as well as substances with moderate and marked activity. One millilitre of 0·025M solution of these substances was injected intradermally immediately after, and in another series immediately before, a dose of 1000-1500r, (60 kV. 10 mA. Filtration inherent in tube shield only, 25 cm. F.S.D. H.V.L. 1 mm. Al.) was given to a circular and a crescent area on the skin of rabbits. Only animals can be used for these tests as some of the protective agents, when injected locally, produce an

inflammatory reaction and the injection on occasion has to be made outside the irradiated area, intradermal diffusion of the agent being relied upon. This was the case, for example, of β-mercaptoaethylamin (Becaptan, Labaz, Horlicks, Ltd.) which was found to be irritating and causing superficial ulceration at the site of injection. Yet it showed its influence on the degree of radiation reaction at a distance in the neighbouring irradiated circle.

Results of 40 experiments are given in Table I. These were undertaken with the intent of testing the method and not of the efficacy of the chemical agents used. The agent in our series which showed unmistakable evidence of protective action was found to correspond on Dr. Alexander's list to the substance (D) with very high protective action in total body irradiation when injected immediately before treatment.

Table I

Protective agent *	Protection	No protection
A. β-mercaptoaethylamin	+ + +	- -
B. Glucose	+ + + + +	-
C. Fructose	+ + +	-
D. Tryptamine hydrochloride	+ + + + + +	-
E. Glycine	+	- - -
F. Thiourea	+	- - - -
G. Urea	+ + +	-
Synkavit		- - -
Hyaluronidase	+	-

* The identity of the substances A to G was learned after the series of experiments was concluded.

Colour photographs of skin reactions will be shown. Data relating to experiments with injection of protector substances before irradiation will be given. The mechanism of dispersal in the connective tissue meshwork of the injected fluids is briefly considered.

The introduction of a skin radiation test for screening of protector substances obviates the difficulties connected with such a process, i.e. study of mortality curves of animals exposed to lethal doses of radiation, laboratory bench assays or of resorting to data based on subjective symptoms of individual patients. It would also offer the advantage of higher local concentrations of substances to be examined and a study of substances which may find an application in our efforts to protect the skin from radiation damage.

Tracing Radioactive Phosphorus Injected Intradermally

It is difficult, and sometimes impossible, to find out by means of directional counters the whereabouts, and the concentration, of radioactive isotopes introduced intradermally. Radioactive phosphorus or zinc or gold colloid is used experimentally for direct infiltration of tumour masses as well as for intraperitoneal and and intrapleural applications. The main requisite is that the injected isotope should remain in situ and only conjectural evidence is available that this is so. On the other hand the

irregular deposition of particles of the isotope or its diffusion along lines of least resistance within the tissue framework is also known. It is believed that the phosphorus remains in situ, especially when suspended in oil, but no direct proof has ever been submitted. A simple method of mapping out on the skin the whereabouts of the radioactive phosphorus injected intradermally has been devised. It consists in giving a dose of 400-800r to the skin over the injected site. It was found that the cumulative effect of radiation due to X-rays, and radiation due to the radioactive isotope will produce a greater skin reaction over areas where both are present, than in those areas where X-rays alone are the reaction-producing agent. Simple erythema and desquamative reaction on the skin often show the distribution of isotopes in a geographical sense. The circle and crescent lead applicator can be used for this purpose when the area to be X-rayed is large, thus reducing the extent of skin reaction.

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EFFECT OF X-RAYS ON THE RESORPTION RATE OF INJECTED BICARBONATE

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In this short communication we present evidence for an x-ray effect which occurs in mice, given a total body irradiation of 2000r, immediately followed by an intraperitoneal injection of $NaH^{14}CO_3$. The mean life-time of the bulk of the circulating bicarbonate ions in the body of the mouse amounting to some minutes only, any change in the rate of resorption will reflect itself in a corresponding change in the amount of $^{14}CO_2$ exhaled already within a few minutes or even seconds after the injection.

In a first set of experiments we started the collection of exhaled carbondioxide about 10 min. after injection of the isotope and followed this process for one hour. We ascertained that irradiated mice exhaled approximately 25 % more $^{14}CO_2$ than controls combined with a slight decrease (some 10 %) in the output of total amount of CO_2. The enhanced exhalation of $^{14}CO_2$ might be expected to run parallel with a smaller amount of residual ^{14}C in the body of irradiated animals. Analyses of controls and irradiated mice showed, however, that the activity of the homogenized and dried tissue did not vary appreciably.

The only explanation left seemed to be that irradiated mice exhale $^{14}CO_2$ at a markedly decreased rate for a very short period immediately after the injection of the bicarbonate, leaving these animals with an increased pool of ^{14}C some 10 - 60 min. afterwards. Experiments to that end, using a special device for collection of CO_2 immediately upon the injection of $H^{14}CO_3$, showed that in the first 4 min of the experiment during which a very appreciable percentage of the injected $^{14}CO_2$ is exhaled, the controls give off more $^{14}CO_2$ (45 %) than the irradiated animals (29 %), and more $^{14}CO_2$ being preserved in the exposed animals they give off more $^{14}CO_2$ in the later stage of the experiment. The activity expired during the first minute, including the 6 sec taken for the injection in these experiments, was found to be only 43 % of that of the control. This indicates that the X-ray effect on the process of resorption of the bicarbonate and the intrusion of CO_2 into the alveolar space is very marked.

The slower exhalation of $^{14}CO_2$ by the exposed animals shortly after injection of the labelled bicarbonate could be due to a depressed resorption or/and a circulation disturbance. To investigate how far the latter is the case we injected labelled bicarbonate into the tail vein of both control and irradiated mice and collected the exhalatory CO_2 in 2 min intervals starting immediately after injection. No conspicuous differences could be noticed in the amount of $^{14}CO_2$ exhaled from irradiated and controls. The depressed exhalation of $^{14}CO_2$ by the exposed mouse has likely been due to a decreased resorption rate of the injected bicarbonate and not to circulation disturbances.

Resorption is presumably mediated through hormonal action. This line of thought induced us to investigate the effect of the administration of various hormones. It appeared that an injection of ACTH effected the exhalation of $^{14}CO_2$ in an opposite way than the irradiation, giving an increased output of some 30% in the first minutes. When irradiating the mice after an ACTH-administration it appears that this combined treatment cancelled the difference between treated and control mice. It may be considered, whether the diminished resorption rate observed in the exposed mouse is due to a blocking effect of the irradiation on ACTH formation. The biological half-life of the injected ACTH is about 5 min and the half-life of the ACTH secreted into the circulation may have a similar value. Blocking of ACTH formation through irradiation would correspondingly soon manifest itself.

Metabolisme de la cystéamine

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Des souris ont reçu 3 mg de S^{35}-cystéamine par injection dans le péritoine. 40 minutes plus tard, on retrouve 50 % de la radioactivité injectée sous forme de cystéamine-cystamine dans l'organisme. 24 heures plus tard, on retrouve dans les tissus 34 % du S^{35} administré, mais 2 % seulement s'y trouvent encore sous forme de cystéamine-cystamine.

Un chien de 7 kg reçoit dans la veine fémorale 104 mg de S^{35}-cystéamine. 16 % du S^{35} injecté sont excrétés en 8 heures: la plus grande partie sous forme de sulfates, 4 % sous forme de cystéamine-cystamine, un peu sous forme de taurine. Sulfates, cystéamine-cystamine et taurine ne rendent pas compte de la totalité de l'activité urinaire; il existe d'autres métabolites marqués qui n'ont pas encore été identifiés.

Histological changes in man and rabbits after parenteral thorium administration

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The most important histological changes which are observed in biopsy material from 8 persons and autopsies from 6 cases are discussed. These are compared with similar material from rabbits who were investigated at different intervals after intravenous administration of colloidal thorium dioxide. In addition is described a disseminated metastasing endotheliosarcoma which is produced in the injected rabbits.

Incorporation du C^{14} dans le glycogène du Foie après Irradiation.

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Des données analytiques montrent qu'on trouve plus de glycogène dans le Foie des animaux irradiés que dans le Foie de témoins jeunant depuis le même temps. (I). Ce fait peut être interprété de deux manières. On peut penser ou bien que l'exposition aux rayons diminue l'utilisation des glucides, ou au contraire qu'elle accélère leur mise en réserve sous forme de glycogene.

Ce problème a été abordé dans ce travail en marquant le glycogène in vivo au moyen de métabolites contenant du C^{14} et en suivant les variations de son activité spécifique.

Les expériences qui ont été réalisées jusqu'à ce jour peuvent être divisées en deux groupes:

I° Dans les expériences appartenant au premier groupe, le métabolite contenant le C^{14} est du glucose et il est injecté lorsque les animaux viennent d'être irradiés. Ce sont des expériences de courte durée: elles sont destinées à suivre l'incorporation dans le glycogène du C^{14} contenu dans le glucose circulant. Elles montrent que chez les animaux irradiés, l'activité spécifique croît plus vite et atteint des valeurs plus élevées que chez les animaux témoins. L'analyse des figures suggère que l'irradiation agit surtout sur la synthèse du glycogène (2).

2° Les expériences du deuxième groupe sont de plus longue durée. Le métabolite qui fournit le C^{14} est du bicarbonate, et il est injecté plusieurs heures ou même plusieurs jours avant que les animaux soient exposés aux rayons.

Ces expériences mettent à profit la remarque suivante: lorsque le glycogène est marqué par du C^{14} provenant du bicarbonate son activité spécifique ne décroit pas d'une manière régulière. Elle subit d'abord une chute rapide, puis remonte et présente un deuxième maximum suffisament élève et surtout suffisament éloigné du moment de l'injection (30 à 40 heures) pour qu'on puisse l'utiliser pour suivre le turn over du glycogène ou sa synthèse à partir de molécules marquées préexistantes alors que les animaux sont revenus à des conditions physiologiques normales.

Ces expériences confirment les premières: elles montrent que l'irradiation agit sur la synthèse du glycogène et non sur sa vitesse de renouvellement.

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THE MODIFICATION OF THE RADIATION RESPONSE BY SHIELDING PROCEDURES

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It has been shown that head shielding or hind-leg shielding of a rat during radiation exposure will modify considerably the response of the animal (Lamerton L.F., Elson L.A. and Christensen W.R: Brit. Journ. Rad., 26, 510, Oct. 1953; Lamerton L.F., Elson L.A. and Harriss E.B: Brit. Journ. Rad., 26, 568, Nov. 1953). The anaemia developing some days after irradiation is much less severe in the case of the shielded animals than in those given whole body irradiation. Two factors which are undoubtedly of considerable importance in the development of the anaemia are (a) the platelet fall following irradiation, and (b) the capacity of the animal for compensatory erythropoiesis. Platelet counts have been made in the case of whole body irradiated and shielded animals and it has been shown that shielding of the head or hind leg will substantially reduce the extent of the platelet fall. The capacity of the animal for erythropoiesis following irradiation has been investigated using techniques involving radioactive iron and it appears that heightened erythropoietic activity is by no means confined to the shielded limb, but that the spleen of the animal plays an important part.

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Hit-theoretical analysis of carcinogenesis by ultra-violet radiation

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Experimental findings show several striking similarities between spontaneous and induced mutations on the one hand and spontaneous and induced carcinogenesis on the other. Thus tumours may be classified, as a rule, in discrete and rather well separated types both as regards morphologic and histologic features and as regards the growth rate. Next, these properties seem, as a rule, to be the same independent of whether the tumour is spontaneous or induced and, in the latter case, independent of the quality and quantity of the agent as well as of the time at which the tumour appears, early or late during extended exposure to a carcinogenic agent.

One of the current theories as to the mechanism of carcinogenesis is, therefore, that this phenomenon is a consequence of a somatic mutation [1]. Thus the present experimental findings indicate that both mutations and carcinogenesis may be thought of as being the results of molecular changes in certain cellular units, these changes occurring spontaneously due to thermal fluctuations, internal chemical agents or cosmic rays, or being induced by external agents, directly or indirectly e.g. via an intermediate radical mechanism. Perhaps in both cases these cellular units or control centres, controlling the phenomena in question, are the genes.

From the hypothesis that carcinogenesis is a consequence of certain molecular changes in certain cellular units, and assuming tentatively for simplicity that (a) the action of the carcinogenic agents is a direct hit on these cellular units, and (b) that one such cellular unit being hit is sufficient to start a tumour[2], we have in a series of papers[3] worked out theoretically the quantitative consequences and compared these predictions with experiments using as carcinogens chemical agents, in the form of hydrocarbons, viruses, and ultra-violet radiation. In the latter case the experiments allow of the most detailed comparison which will be illustrated in a number of slides during the reading of this paper. The agreement

between the theory in its tentative form and the experiments is satisfactory, but indicate that in any case with ultra-violet light as carcinogenic agent the action may be an indirect one, presumably via a radical mechanism. Further experiments, investigating e.g. a possible oxygen effect on carcinogenesis, are therefore highly desirable to elucidate the mechanism of carcinogenesis.

1) Cf.e.g. p. 444 ff in "Symposium on Radiobiology" ed. by J.J. Nickson, John Wiley and Sons, New York 1952.
2) cf. loc.cit. p.242 ff.
3) "On the mechanism of experimental carcinogenesis", Acta Path. et Microbiol.Scand. :
I Iversen and Arley (general theory) vol.XXVII p.773, 1950
II Engelbreth-Holm and Iversen: The effect of different concentrations of 9, 1o-dimethyl-1,2-benzanthracene on skin-carcinogenesis in mice. vol.XXIX, p.77, 1951
III Arley and Iversen: Further development of the hit theory of carcinogenesis. vol.XXX, p.21, 1952
IV Iversen and Edelstein: The early mitotic effect of 9,1o-dimethyl-1,2-benzanthracene and of 1,2,5,6-dibenzanthracene on epidermal cells of the mouse ear. vol.XXX, p.213, 1952.
V Iversen and Arley: Application of the hit theory to virus-induced tumours. vol. XXXI, p.27, 1952
" cf. also Arley and Iversen: Application of the quantum hit theory to virus-provoked tumours. NATURE vol.169, p.410, 1952
VI Arley and Iversen: Hit theoretical interpretation of some experiments of Berenblum and Shubik. vol. XXXI, p.164, 1952

VII Iversen and Engelbreth-Holm and Noring: On the effect of croton oil on skin previously painted. vol. XXXII, p.218, 1952
VIII Ringsted: The growth of Mill-Hill endothelioma in chicks treated with aminopterin. vol. XXXIII, p.44, 1953
IX Arley and Iversen: Application of the hit theory to tumours produced by ultraviolet radiation. vol. XXXIII, p.133, 1953
" cf. also Iversen and Arley: Application of the quantum hit theory to tumours induced by ultraviolet radiation. NATURE vol. 171, p.257, 1953
X Engelbreth-Holm and Jensen: The influence of age and on growth hormone on skin carcinogenesis in mice. vol. XXXIII, p.257,1953

Summary of Observations made on the Human Response to a Single Dose of X-rays

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Since 1950 the constitutional disturbance produced by a single dose of X-rays has been studied in 150 patients. These investigations have as far as possible been carried out under controlled conditions, though only patients in good general health and those willing to co-operate have been studied.

Human investigations of this type are limited by ethical and therapeutic considerations in respect of the dose of X-rays that can be given and the volume of tissue that can be irradiated. Within these limits standardised studies have been made on the effects of middle voltage X-irradiation of the pelvis, whole abdomen, whole length of spine, upper half of the trunk and to a limited extent of the whole body.

The main lines of study have been the nature and time of development of the initial symptoms, together with the associated changes in the peripheral blood count, in the electrolyte excretion and renal function, and in the vascular response within the irradiated volume. In addition, observations on adrenal function have been made during and prior to the period of symptomatic disturbance.

Body Size and Dose Relationship to the Initial Symptomatic Disturbance of Radiation Sickness.

After the administration of a large enough X-ray dose to either the whole body or to an adequate volume of the trunk, there is an asymptomatic or "latent" period terminated by the sudden onset of symptoms and signs of radiation sickness. These are nausea, headache, fatigue and often vomiting. The latent period from the commencement of irradiation to the onset of symptoms has been shown to be dependent on the administered radiation dose in roentgens, the body size, and a particular property of the site irradiated, the anatomical site sensitivity. The latent period is not, however, dependent on whole body integral dose.

There is, for any given site irradiated, a linear relationship between the response metameter - in this case latent period - and the log dose per unit of body size. This relationship strongly suggests that the time of onset, duration and severity of the initial disturbance are largely determined by the release of a diffusible substance in the irradiated volume with subsequent dilution throughout one or more of the fluid compartments of the whole body; symptoms then occur if and when a particular threshold is reached. In other words we appear to be dealing, at least in part, with a typical pharmacological dose-response relationship.

Peripheral Blood Count Response

Qualitatively the changes in the blood count are similar to those noted after single whole body exposures in animals, although the doses used are not great enough to produce clinical anaemia.

A neutrophil leucocytosis accompanies the onset of symptoms and may precede it. The maturity of the neutrophils suggests that the cells are mobilised from vascular channels opened up as a result of irradiation. The changes in white count at this time are not characteristic of those induced by ACTH, epinephrine or histamine.

In a group of cases given irradiation to the whole length of the spine, a mean dose of the order of 200r has been delivered to the spinal bone marrow. Subsequently this group has shown a significant fall in the lymphocyte count from the first post-irradiation day, becoming maximal about the seventh day, a significant fall in the white cell count from the second post-irradiation day, but no significant fall in the neutrophil count until the fifth day.

In a high proportion of cases the venous haematocrit falls from the first, second or third post-irradiation days. It is likely that at such an early stage this fall is due either to a change in plasma volume, consequent upon a redistribution of intra-cellular and extracellular fluid, or to increased red cell haemolysis.

Electrolyte Excretion and Renal Function

The onset of symptoms is usually associated with changes in the excretion of electrolytes. There is an increased excretion of sodium ion, the increase being mainly covered by increases in the excretion of phosphate and chloride ions. Although some increase may occur in the glomerular filtration rate as determined by changes in creatinine clearance, this is insufficient to explain the change in sodium excretion. Within the limits of errors of estimation, the plasma sodium and inorganic phosphorus levels do not appear to change, and calculations of the tubular rejection factor for these ions suggests that there is interference with their back transport through the cells of the proximal renal tubules. It is difficult, however, to distinguish between changes which may be due to the effects of radiation on tubular function and changes in electrolyte excretion which may result from the natural homeostatic functions of the kidney.

Adrenal Function

Tests of adrenal function have so far failed to demonstrate any disturbance at the time of or prior to the development of radiation sickness. These tests include estimations of 17-ketosteroid excretion, the plasma compound F level and the absolute eosinophil count.

The Vascular Response in Irradiated Tissue

The rate of clearance of intra-dermally injected radiosodium has been used to investigate the effect of irradiation on the vascularity of irradiated skin and presumably of the vascularity of tissues within the irradiated volume. Increases in the radiosodium half clearance time after intra-dermal injection have been found with X-ray doses in excess of 500r. These changes appear within $1\frac{1}{2}$ to 2 hours of irradiation and may be maximal prior to the appearance of a visible erythema. Further investigations are being undertaken to determine the relationship of these changes to radiation sickness and to changes in the neutrophil count already described or occurring towards the end of the latent period.

Conclusion

Whatever the mechanism of the changes observed and the clinical disturbance produced, the clear relationship between response, measured as latent period, and log dose per unit of body size, provides a reproducible biological system. This system is moreover a convenient means of assessing the efficiency of either protective or therapeutic agents by established pharmacological methods of bio-assay on man himself.

Relative biological efficiency of fast neutrons and gamma radiation for chronic irradiation of mice.

by R.H. Mole., R.J. Munson and G.J. Neary.

A graphite pile at A.E.R.E. Harwell has been used for daily irradiation of mice with fast neutrons substantially free from gamma radiation. Sterilisation of male and of female mice and a reduction in the weight of the testis have provided sensitive indicators of radiation damage. Dose-response curves for fast neutrons and Cobalt gamma radiation have been found to be very similar: there has been no evidence so far of any qualitative difference. Relative biological efficiency factors lay between 3 and 8 for exposures ranging from twentyfour hours to five months: there are definite differences between different strains of mouse. An experiment to determine the r.b.e. for lethal effects of low daily irradiation has been started but no results are yet available.